REMARKS/ARGUMENTS

Claims 1-26 have been canceled and claims 27-33 are currently pending.

Table 2

Table 2 has been amended to include DH10B(pKmAT, pUC19) and DH10B(pKmAT, pRV380) as described in Example 7, page 12. The pKmAT plasmid contains an acetyl transferase as described in Vidali, *et al., Applicability of CoA/acetyl-CoA manipulation system to enhance isoamyl acetate production in Escherichia coli*, Met. Eng. 6:294-9, at Table 2 (2004) (showing that pKmAT contains ATF). The pUC19 plasmid is a control vector and pRV380 is a PANK expression vector as described in Table 1.

Δ*ackA* or Δ*pta* is NOT new matter

Claim 27 is rejected as lacking a written description for Δ*ackA* or Δ*pta*, instead only describing Δ*ackA-pta*. Yet the application describes the *ackA* and *pta* genes as separate genes in the same operon, see p. 5 ¶ [30], p. 14 ¶ [56], and p. 15 ¶ [57]. The YBS121 (Δ*ackA-pta*) is not a "mutant" strain rather it is a strain generated by chromosomal integration that has "mutant copies acetate kinase (*ackA*) and phosphoacetyltransferase (*pta*)." p. 15 ¶ [57]. Thus each gene *ackA* and *pta* was intentionally mutated to generate the double-mutant YBS121. Therefore, Applicants describe Δ*ackA* and Δ*pta*. Either Δ*ackA* or Δ*pta* could be intentionally mutated or, as one example, both could be intentionally mutated.

Ester Production is NOT new matter

The Examiner argues that production of any ester was introduced after the final amendment. Applicants note that the original claims filed on March 24, 2004 describe production of any compound increased by elevated expression of A-CoA metabolism, especially claims 1-5, 10, and13. Original claim 13 specifically describes target compounds "succinate, isoamyl alcohol, isoamyl acetate, **esters**, PHBs and polyketides" (emphasis added). Applicants elected claims 1-5 in response to restriction directed to methods of increasing A-CoA metabolism by elevating PANK, ATF and PDH. Original claims 1-5 do not specify any one product produced by increased CoA metabolism. Claims 27-33 were drafted in response to the

Final Restriction and specify a bacterial cell with recombinant PANK, recombinant PDH and recombinant ATF. Only claim 32 and dependent claim 33 specify production of isoamyl acetate. Claims 27-31 are directed to increased A-CoA flux through recombinant PANK, PDH, and ATF. Therefore the claims are not directed to solely isoamyl acetate production, rather describe one embodiment as isoamyl acetate production out of many target compounds that including esters.

Enablement

The Examiner states that a bacterial cell transformed with recombinant PANK, PDH, and ATF is not enabled and that only an E. coli cell transformed with PANK, PDH, and ATF is enabled. Applicants strongly disagree. Gene expression in a plethora of bacteria is an established protocol with tables of codon usage publicly available. No experimentation is required to identify a gene of interest, convert the codons to those used by a desired organism, and express the protein in that organism. The National Center for Biotechnology Information (NCBI), used frequently by those of ordinary skill in the art and publicly available from the United States National Library of Medicine, describes codon usage in organisms from humans to viruses, including Bacteria.

The attached Declaration of Dr. George N. Bennett states: A) Coenzyme A synthesis and glycolytic pathways are conserved across all bacteria; B) expression of PANK, PDH and ATF will increase A-CoA flux in all bacteria; and C) PANK, PDH and ATF are defined. Thus expression of recombinant PANK, PDH, and ATF will increase A-CoA flux in any bacterial cell. One of ordinary skill in the art can easily and without ambiguity identify enzymes with pantothenate kinase, pyruvate dehydrogenase and alcohol acetyl transferase activity, express those genes in a bacteria using standard protocols, and those bacteria will increase A-CoA flux. Applicants provide an example of PANK, PDH, and ATF expression in cell strain YBS121 (pATCA, pGS367) that expresses recombinant PANK, PDH, and ATF (Table 2) in a Δ*ackA* and Δ*pta* strain as described in example 11 and 12. This is provided as one embodiment of the invention and not intended to limit examination to one of many embodiments. Thus Applicants have provided a generated bacterial cell with a recombinant PANK, PDH and ATF and methods of increasing Co-A flux as demonstrated in FIG 10.

Obviousness

Claims 32 and 33 are rejected as obvious over San in view of Vallari, Voet and Yang. However, Applicants note that no art rejection is made against claim 27, and that claim 32 has all of the elements of claim 27, plus additional elements. Thus, if claim 27 is free of the art, so should claim 33 be free of art. However, in the event that the failure to reject claim 27 was inadvertent, Applicants will address the substance of the rejection as well.

The Examiner is directed to MPEP §2142 Legal Concept of *Prima Facie* Obviousness. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) **must teach or suggest all the claim limitations.**

The Cited Art Does Not Teach All the Claim Limitations

The claimed invention relates to bacteria having recombinant PANK, PDH and ATF added thereto, and growth of the bacteria in a medium supplemented pantothenic acid, among other things. The cited references do not recite "recombinant PANK," "recombinant PDH," or supplementation "with pantothenic acid."

San teaches two bacteria having exogenous ATF and differing activity of endogenous PANK. San **does not** teach bacteria having recombinant PANK, recombinant PDH or reduced activity of *ackA* or *pta*.

Vallari teaches the endogenous PANK strains used in San, above. Vallari **does not** teach a bacteria having recombinant PANK, recombinant PDH or reduced activity of *ackA* or *pta*.

Yang teaches Δ*ackA-pta-nuo*. Yang does not teach a bacteria having recombinant PANK, PDH or ATF.

Voet does not teach bacteria having **any** recombinant genes, only basic metabolic pathways. Voet teaches a plethora of basic metabolic pathways including A-CoA formation, but

does not teach which biochemical pathways are amenable to genetic manipulation, expression in recombinant systems, or methods of using the pathways to modify bacterial metabolism. The fact that the claimed invention is within the capabilities of one of ordinary skill in the art is not sufficient by itself to establish *prima facie* obviousness MPEP 2143.01 ¶IV.

The cited references do not teach supplementation with pantothenoic acid. Therefore, the cited references together teach exogenous PANK with ATF can produce isoamyl acetate from isoamyl alcohol. The cited references do not teach generated bacteria with recombinant PANK, PDH, and ATF cultured with pantothenic acid to increase CoA flux. The cited references do not provide methods to increase CoA flux "relative to" the currently claimed bacteria with recombinant PANK, PDH, and ATF and supplementation with pantothenic acid and as taught in examples 11 and 12.

Secondary Indications of Non-obviousness

As noted by Vadali, et al. (Metab. Eng. 6:133-9 (2004), at page 138)[1], "It was found that the intracellular CoA/acetyl CoA could be increased **only** with the simultaneous overexpression of pantothenate kinase **and supplementation of pantothenic acid**. Since *E. coli* normally secretes out excess pantothenic acid, it might be logical to assume that the availability of precursor will not be rate limiting. On the contrary, the **supplementation of pantothenic acid is essential and necessary** for CoA/acetyl-CoA manipulation." (*emphasis added*) Thus at the time of filing, it was unexpected that bacteria would require supplementation with pantothenic acid and, without supplementation, increased CoA flux would not be achieved. One of ordinary skill in the art would not have predicted without this research as published in Vadali and presented in the current application "cofactor manipulation and carbon flux enhancement are synergistic and much more effective in increasing isoamyl acetate production, than using either of the strategies alone," P.16 ¶[59].

Even if the art did teach every recited element (and it does not), it is not obvious to make the combination suggested by the Examiner. As admitted by the Examiner at page 3 of the

[1] Metabolic Engineering is respected and **peer reviewed** journal with an Initial Impact Factor of 3.397 (www.elsevier.com/framework_editors/pdfs/Perspectives1.pdf).

Office Action, "It is **not** clear to one having skills in the art, why the Applicants, aiming at the increase in CoA production in a transformed cell, transfect the cell with a [PDH] gene and with ATF2 gene. According to the state of the art at the time of filing, **both enzymes deplete the CoA pool.**" Therefore, there is no motivation to make the suggested combination.

Yang, et al. (1999) **teaches away** from being able to predict metabolic engineering *de novo* based on a desired product. Yang states, "The drastic effect of a *nuo* deletion in the *ackA-pta* background was counterintuitive." A shift to lactate formation and a dramatic decrease in the flux through the PFL pathway was not predicted. In the present application, it could not have been predicted that "cofactor manipulation and carbon flux enhancement are synergistic and much more effective in increasing isoamyl acetate production, than using either of the strategies alone," P.16 ¶ [59]. Therefore, at the time the application was filed, the effect of recombinant PANK, PDH, and ATF in bacterial cells supplemented with pantothenic acid was not predictable.

CONCLUSIONS

The claims clearly describe the claimed invention and the specification enables one of ordinary skill in the art to use the present invention. The cited art does not teach bacteria with recombinant genes encoding PANK, PDH **and** ATF, or "culturing said cell in a cell medium comprising **pantothenic acid**."

Each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue. At the very least, claim 27 and its dependant claims appear to be free of the art and 112 rejections. Therefore, Applicants request allowance of these claims. Applicants respectfully request the Examiner contact them if there are any questions or issues that need to be addressed.

The fees for a two month extension and request for continued examination are enclosed. It is believed that no additional fees are required for this submission. Should Applicants be incorrect, please charge additional fees and credit any overpayment to Deposit Account No. 50-3420 (reference 31175413-005002 MDB)

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Respectfully submitted,

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